Chapter

Heterogeneity of Fibroblasts in Healthy and Diseased Kidneys

Takahisa Yoshikawa, Yuki Sato and Motoko Yanagita

Abstract

Chronic kidney disease (CKD) is a worldwide health problem affecting 9.1% of the world's population. The treatments to prevent the progression of CKD remain limited, however. Resident fibroblasts in the kidneys play crucial roles in the pathological conditions commonly recognized in CKD, such as renal fibrosis, renal anemia, and peritubular capillary loss. Fibroblasts in the kidney provide structural backbone by producing extracellular matrix proteins and produce erythropoietin for normal hematopoiesis under physiological conditions. In the diseased condition, however, fibroblasts differentiate into myofibroblasts that produce excessive extracellular matrix proteins at the cost of the inherent erythropoietin-producing abilities, resulting in renal fibrosis and renal anemia. Pericytes, which are mesenchymal cells that enwrap peritubular capillaries and highly overlap with resident fibroblasts, detach from peritubular capillary walls in response to kidney injury, resulting in peritubular capillary loss and tissue hypoxia. Several reports have demonstrated the beneficial roles of fibroblasts in the regeneration of renal tubules Renal fibroblasts also have the potential to differentiate into a proinflammatory state, producing various cytokines and chemokines and prolonging inflammation by forming tertiary lymphoid tissues, functional lymphoid aggregates, in some pathological conditions. In this article, we describe the heterogenous functions of renal fibroblasts under healthy and diseased conditions.

Keywords: chronic kidney disease, erythropoietin, renal anemia, fibrosis, myofibroblast, tertiary lymphoid tissue

1. Introduction

Chronic kidney disease (CKD) is a worldwide public health problem. In 2017, the prevalence of CKD was estimated to be 9.1% in the world's population, and has increased by 29.3% from 1990 to 2017 [1]. The prevalence of CKD in elderly individuals over 65 years old is especially high and is predicted to increase further as a result of the increasingly aged society [2]. CKD is a risk factor for end-stage renal disease (ESRD) and is also recognized as an independent risk factor for cardiovascular diseases and their associated mortality [3]. Patients with ESRD need renal replacement therapies such as dialysis and renal transplantation to survive. The cost of these therapies is enormous and the financial burden is a critical problem for patients and society [4]. Nevertheless, treatment to prevent the progression of CKD and the occurrence of CKD-associated complications remain limited.

Fibroblasts are distributed in various organs throughout the body and contribute to both homeostasis and disease. In the kidney, resident fibroblasts play crucial roles in both health and disease, and their phenotypes are heterogenous and plastic [5]. Under physiological conditions, renal fibroblasts provide structural support for the entire kidney architecture and produce erythropoietin (EPO). In contrast, in diseased kidneys, fibroblasts lose these physiological functions and transdifferentiate into myofibroblasts. These phenotypic changes result in fibrosis, renal anemia, and peritubular capillary loss, all of which are common pathological conditions of CKD, irrespective of the etiology [6]. Renal fibroblasts also act as inflammatory cells and produce proinflammatory cytokines and chemokines under some pathological conditions [5, 7]. In aged injured kidneys, fibroblasts play a crucial role in prolonging inflammation by inducing tertiary lymphoid tissue (TLT) formation [8]. These features highlight the importance of understanding the behavior of fibroblasts in the kidney in order to identify efficient therapeutic strategies to prevent CKD progression. In this article, we describe the current understanding of the heterogeneous functions of fibroblasts in healthy and diseased kidneys.

2. Fibroblasts in the kidney

2.1 Characteristics and functions of resident fibroblasts in kidneys

Renal resident fibroblasts are spindle-shaped cells that exist in the interstitial space, which is defined as the area between nephrons. Nephrons are functional units of the kidney and are composed of glomerular and tubular cells. Fibroblasts provide the structural backbone of the kidney by producing extracellular matrix (ECM) proteins and interact with surrounding cells to maintain the homeostatic state in healthy kidneys. Identification of resident fibroblasts is performed based on their location, shape, and positive expressions of several fibroblast markers such as CD73 and PDGFRβ [9]. As these markers are neither homogeneously positive nor specific for resident fibroblasts, confirming the negative expression of other cell-lineage markers such as CD45, a hematopoietic cell marker, is also necessary to identify renal resident fibroblasts.

In addition to the role of structural cells, fibroblasts have unique organ-specific functions. In the kidney, small subset of the renal resident fibroblasts residing in the corticomedullary area produces Epo, a hormone essential for erythropoiesis in response to hypoxia [10]. Although there are few Epo-producing cells and they exist only in the deep cortex in the physiological state, under severe hypoxic conditions such as severe anemia, the number of Epo-producing cells increases and they can be detected in the cortical area [11]. The increase in the number of Epo-producing cells under hypoxic conditions is likely due to the increase in the number of the cells that have acquired Epo-producing ability but not to cellular proliferation, because Epo production is activated in anemic mice under administration of a cell cycle inhibitor or γ-ray irradiation [10]. Interestingly, while other growth factors for hematopoietic cells (such as granulocyte colony-stimulating factor) are produced in bone marrow, where hematopoietic cells are generated and the growth factors are required, EPO is produced from the kidney. One possible explanation for this is that kidneys are physiologically hypoxic compared with other organs, which allows them to be more sensitive to small changes in oxygen delivery than other organs and is advantageous to the production of EPO in response to hypoxia [12]. Another explanation is that kidneys function as a

"critmeter," with the ability to set hematocrit within the normal range by regulating plasma volume and the red blood cell mass in a common site [13].

2.2 Origin and heterogeneity of fibroblasts in the kidney

In 1974, Le Douarin et al. reported that, in transplantation experiments of quail neural tubes to chicks, quail neural crest–derived cells were identified in the renal interstitial space [14]. Consistently, we found that fibroblasts in neonate kidneys express p75 neurotrophin receptor (p75NTR), a neural crest marker [15]. Moreover, Epo-producing cells express neuronal markers such as microtubule-associated protein 2 and neurofilament light polypeptide [10]. Based on these previous findings, we conducted a lineage tracing study using *myelin protein zero (P0)-Cre* mice, which label neural crest–derived cells, and found that resident fibroblasts in the cortex and corticomedullary area are lineage-labeled with *P0-Cre* [15]. We also demonstrated that, in embryonic kidneys, *P0-Cre* lineage–labeled cells appeared in the interstitial space along the outer capsule and ureter from E13.5 and more than 99% of the resident fibroblasts in the cortex and outer medulla were labeled with *P0-Cre* in adult kidneys. Consistently, Epo-producing fibroblasts were also labeled with *P0-Cre* in the experiment using *P0-Cre:R26R:EPO-GFP* mice [15]. In contrast, renal fibroblasts in the medulla are not labeled with *P0-Cre*. Wnt4 expression is identified in the medullary stromal mesenchyme in embryotic kidneys and reactivated in medullary fibroblasts after renal injury [16, 17]. These findings suggest the regional heterogeneity of fibroblasts and their origin in the adult kidney. The functional heterogeneity of PDGFRβ$^+$ Epo-producing cells was also reported. Precise histological analysis showed that different subpopulations of fibroblasts produce Epo responding to different stimuli [18].

2.3 Pericytes in kidneys

Pericytes are mesenchyme-derived cells that enwrap capillaries with their processes embedded in the vascular basement membrane. Resident fibroblasts and pericytes share several characteristics, including their interstitial location and cell surface markers such as CD73 and PDGFRβ, and, as such, these two types of cells are often confused. Pericytes support the capillary structure and regulate vascular tone with their contraction force [19]. Moreover, they interact with endothelial cells to maintain capillary homeostasis [6]. Humphreys et al. reported that the origins of pericytes in the kidneys were FoxD1-expressing cells in an experiment using *FoxD1-Cre* mice [20]. On the other hand, it was reported that *P0-Cre* lineage–labeled cells in E13.5 embryonic kidneys transiently expressed FoxD1 [15]. Based on these findings, it is assumed that resident fibroblasts and pericytes are highly overlapped populations in the kidneys.

3. Renal fibrosis as a hallmark of CKD

Renal fibrosis is a common pathological condition of CKD, irrespective of the etiology. It is defined as excessive accumulation of ECM such as collagen and fibronectin in the interstitial space and is recognized as a predictive indicator of renal prognosis [21]. Previous studies have shown that dysfunction of the renal fibroblasts can induce several pathological conditions associated with CKD, such as renal fibrosis, renal anemia, and peritubular capillary loss. Against this background, renal fibroblasts have been focused on as hopeful therapeutic targets for CKD and its complications.

3.1 Myofibroblasts in kidneys and their origin

Myofibroblasts are recognized as the main contributor to fibrosis in multiple organs. They are characterized by dense endoplasmic reticulum and contractile microfilament bundles [22]. Their most prominent feature is the expression of α-smooth muscle actin (α-SMA) that forms myofilament bundles and promotes their high contractility [23]. Although myofibroblasts are almost undetectable in healthy kidneys, they expand dramatically in diseased kidneys and drive fibrosis by producing a large amount of ECM proteins and through their own proliferation. The origin of myofibroblasts has been discussed for decades, and several genetic lineage tracing studies recently revealed that resident fibroblasts and pericytes are the main sources for myofibroblasts [9]. We reported that *P0-Cre* lineage–labeled cells, which were progenitors of resident fibroblasts (as mentioned above), could differentiate into myofibroblasts in several kidney injury models [15]. Humphreys et al. reported that *FoxD1-Cre* lineage–labeled pericytes are the main sources for myofibroblasts [20]. These studies demonstrated that most myofibroblasts are derived from these renal fibroblasts and pericytes. Although several studies have reported other types of cells as progenitor cells for myofibroblasts, such as epithelial cells, endothelial cells, and hematopoietic cells, recent lineage tracing experiments demonstrated that tubular epithelial cells do not become myofibroblasts *in vivo* [20, 24, 25]. The endothelial-to-mesenchymal transition, in which endothelial cells transdifferentiate into myofibroblasts, was also reported to contribute less to myofibroblast pools than resident fibroblasts [24]. Additionally, Kramman et al. used single-cell RNA sequencing (scRNA-seq) and parabiosis techniques to demonstrate the limited contribution of circulating monocytes to myofibroblast pools with very few matrix genes expression in murine fibrotic kidneys [25].

Notably, although genetic lineage tracing is not feasible in humans, a recent study utilizing scRNA-seq of human kidney samples supports the notion that these findings in mice appear to be conserved in humans. Kuppe et al. conducted scRNA-seq on human kidneys in patients with CKD and demonstrated that Notch3$^+$ pericytes and Meg3$^+$ fibroblasts were the main sources for highly ECM-producing myofibroblasts using pseudo-time trajectory analysis and diffusion map analysis [26]. These studies support the idea that most renal myofibroblasts derive from renal resident fibroblasts or pericytes.

3.2 Progenitor of myofibroblasts; Gli1$^+$ fibroblasts in the perivascular niche

Mesenchymal stem cells (MSCs) are defined as cells with self-renewal and clonogenic capacity. Gli1$^+$ fibroblasts are MSC-like cells that reside in both the pericyte niche and the adventitia of larger vessels across multiple organs, including the kidney, and exhibit trilineage differentiation potential *in vitro* [27, 28]. A fate tracing study utilizing *Gli1-CreERt2:tdTomato* reporter mice revealed that, although Gli1$^+$ fibroblasts represented only 0.2% of the PDGFRβ$^+$ renal fibroblast population in healthy kidneys, after renal injury, they proliferated dramatically, mainly in the medulla and inner cortex, and differentiated into αSMA$^+$ myofibroblasts. Additionally, using *Gli1-CreERt2:iDTR* (inducible diphtheria toxin receptor) mice, the ablation of Gli1$^+$ cells by diphtheria toxin (DT) administration dramatically reduced renal fibrosis by approximately 50% after unilateral ureteral obstruction (UUO), which is an *in vivo* experimental model of renal fibrosis. These data suggested that Gli1$^+$ fibroblasts predominantly proliferated and contributed to renal fibrosis, suggesting the heterogeneity of the potential to transdifferentiate into myofibroblasts among PDGFRβ$^+$ fibroblasts in the kidney.

3.3 The roles of proximal tubule injury in CKD progression

Acute kidney injury (AKI) is a highly prevalent disorder and is one of the risk factors for the progression of CKD [29]. The underlying molecular mechanisms for CKD transition after AKI have been investigated for decades. The proximal tubules are the most vulnerable segment in the nephron, and are assumed to trigger the AKI to CKD progression. To investigate whether injured proximal tubules can trigger renal fibrosis, we selectively damaged proximal tubules by DT administration in *Ndrg1-CreERt2:iDTR* mice, in which DTR is specifically expressed on proximal tubules in the kidneys [30]. Low-dose single DT administration caused mild proximal tubule injury and reversible fibrosis whereas high-dose single DT or repeated low-dose DT administration caused sustained renal fibrosis. This study showed that injury of the proximal tubules is sufficient to cause several features of CKD, and that the frequency and severity of proximal tubule injury are associated with the degree of AKI to CKD progression. As an explanation for the association between proximal tubule injury and CKD, tubulointerstitial interactions in injured kidneys have been reported to contribute to renal fibrosis [31]. Yang et al. demonstrated that, in a multiple profibrotic AKI model, injured proximal tubules underwent cell cycle arrest in G2/M and acquired the profibrotic secretory phenotype by upregulating profibrotic cytokine production, such as transforming growth factor β-1 (TGFβ-1) and connective tissue growth factor [32]. Additionally, the administration of a p53 inhibitor, a therapy employed for bypassing G2/M arrest, attenuated renal fibrosis in a renal ischemic reperfusion injury model. Injured proximal tubules have been reported to secrete several other profibrotic ligands expressed during renal development. The Wnt family plays a crucial role in kidney development, and many Wnt family genes are upregulated in fibrotic kidney models [33]. Zhou et al. demonstrated that the selective ablation of Wntless, a cargo receptor necessary for Wnt secretion, in renal tubular epithelial cells but not in interstitial fibroblasts attenuated renal fibrosis in a UUO model, suggesting that the Wnt family secreted by renal tubules contributed to renal fibrosis. Moreover, Maarouf et al. showed that Wnt1 expression genetically induced in proximal tubules was sufficient for renal fibrogenesis by inducing interstitial myofibroblast activation and proliferation [34]. According to these reports, one of the mechanisms of the AKI to CKD transition is that ligands secreted from injured renal tubules contribute to renal fibrogenesis by activating myofibroblasts.

4. Two common CKD complications, renal anemia and peritubular capillary loss, are also caused by dysfunction of renal fibroblasts/pericytes

Renal anemia is a common complication that affects the majority of patients with CKD [35]. The cause of renal anemia is the relative deficiency of EPO. Several recent studies have shown that dysfunction of renal fibroblasts contribute to this complication. EPO production is stimulated by hypoxia and regulated by hypoxia-inducible factors (HIFs). In normoxic conditions, HIFs are hydroxylated by HIF-prolyl hydroxylase domain–containing proteins (PHDs), and hydroxylated HIFs are degraded by the ubiquitin-proteasome system [36, 37]. In hypoxic conditions, the hydroxylation and degradation of HIFs is inhibited, resulting in the transcriptional activation of HIF-inducible genes, including *EPO*. In renal fibrosis models, the Epo-producing fibroblasts transdifferentiate into myofibroblasts in response to kidney injury and decrease the capacity to produce Epo at the same time [11, 15, 38]. Souma et al. demonstrated that activation of HIFs by the genetic

inactivation of PHDs in Epo-producing cells restored Epo production in Epo-producing cell–derived myofibroblasts in a renal fibrosis model [39]. Additionally, severe anemia or the administration of selective estrogen receptor modulators, the neuroprotective agents, neurotrophin, and the renoprotective agent, hepatocyte growth factor, restored the ability to produce Epo in myofibroblasts [15]. These results demonstrated that the ability to produce Epo in myofibroblasts has plasticity and can be restored by therapeutic interventions. As for the mechanism of the decrease of Epo production in myofibroblasts, Souma et al. showed that NFκB signaling repressed Epo production in fibroblasts in UUO models. Moreover, *Epo-Cre:R26-IKK2ca/+* mice, in which NFκB signals in Epo-producing cells were selectively activated, showed that 20% of Epo-producing cells were positive for α-SMA, suggesting that NFκB signaling also contributed to the transition of EPO-producing fibroblasts into myofibroblasts [11]. Another possible mechanism for the repression of Epo production in myofibroblasts is that hypermethylation in the *Epo* promotor, which is induced by TGFβ-1 stimulation, inhibits *Epo* expression in myofibroblasts in the fibrotic kidney [38]. Fuchs et al. also showed that Epo production in renal fibroblasts was suppressed by TGFβ signaling in renal fibrosis models, utilizing *PDGFR*β*-Cre:TGF*β*-R2*$^{fl/fl}$ mice, and hypothesized that it occurred before the phenotypic shift of fibroblasts to myofibroblasts because the frequency of α-SMA^{+} myofibroblasts did not differ between the knockout mice and control mice [40].

Although the administration of erythropoiesis-stimulating agents (ESAs) is a currently well-established and effective clinical treatment, it might be associated with several adverse effects, such as hypertension and thrombotic complications [41]. To avoid safety concerns associated with ESAs, PHD inhibitors, which upregulate EPO production via the stabilization of HIFs, have been developed and used for the treatment of renal anemia [36, 42–45].

Another common pathological feature of CKD is the loss of peritubular capillaries [46]. Renal pericytes enwrap peritubular capillaries and support them structurally. In response to injury, pericytes detach from capillaries and their processes, which form networks surrounding the capillaries, start to direct from their associated capillaries to the adjacent tubules, concomitant with transdifferentiation into myofibroblasts [39]. This pathological change makes peritubular capillaries unstable and causes capillary rarefaction and loss [47]. Reduced peritubular capillary blood supply can cause chronic hypoxia in renal parenchymal cells such as tubules and stromal cells. Hypoxia aggravates renal fibrosis by stimulating fibroblasts and altering their gene expressions associated with ECM metabolism [48]. For example, hypoxia upregulated collagen type 1 and the tissue inhibitor of metalloproteinase-1 expression and also downregulated matrix metalloproteinase-1 *in vitro*, which led to the accumulation of ECM proteins. Excessive fibrosis also reduces the efficiency of oxygen diffusion due to the expanded distance between capillaries and tubules and induces renal damage, which can drive further CKD progression. Recently, a sodium-glucose cotransporter 2 (SGLT2) inhibitor, which prevents glucose reabsorption by SGLT2 in proximal tubules, was reported to prevent tissue hypoxia and renal fibrosis in an ischemic reperfusion injury (IRI) model by ameliorating renal capillary rarefaction and the detachment of pericytes from endothelial cells with the promotion of vascular endothelial growth factor-A expression in proximal tubular cells [49]. Additionally, SGLT2 inhibitor treatment was reported to increase hematocrit concomitantly with the elevation of serum EPO concentrations in patients with type 2 diabetes mellitus [50, 51]. On the basis of these reports, SGLT2 inhibitors are expected to play a beneficial role in the restoration of the physiological functions of fibroblasts.

5. Beneficial function of myofibroblasts in kidneys

Contrary to the long-held assumption that fibrosis is detrimental to the host, recent evidence suggests that fibrosis also has host-protective roles in some cases. To investigate the role of fibroblasts during the early phase of kidney injury, we utilized *P0-Cre:iDTR* mice [52], which allow us to induce resident fibroblast-specific dysfunction at the desired time point by DT administration. Utilizing this system, we found that the dysfunction of resident fibroblasts in the acute phase of injury impaired tubular regeneration. During the transition from fibroblasts to myofibroblasts, fibroblasts upregulate the expression of retinaldehyde dehydrogenase 2 (RALDH2), a rate-limiting enzyme in retinoic acid (RA) synthesis. Given that RAs are essential for kidney development, RAs derived from myofibroblasts might promote tubule regeneration. Retinoic acid receptor (RAR) γ and the downstream molecules such as αB-crystallin were expressed in proximal tubules in injured kidneys, and the administration of an RAR inverse agonist to the proximal tubule cell line attenuated proliferation *in vitro*. Another example of the beneficial role of fibroblasts during injury was shown by the experiment utilizing the intravital imaging method [53]. Schiessl et al. demonstrated that, in response to laser-induced tubular cell injury, PDGFRβ^{+} interstitial cells migrated towards tubular injury sites and enclosed the injured tubules. Additionally, PDGFRβ inhibitors compromised the recruitment of the interstitial cells and tubule regeneration. These results suggest that fibroblasts have the potential to promote tubule regeneration, at least in the acute phase of kidney injury, via PDGFRβ signaling.

6. Renal fibroblasts associated with inflammation

Although myofibroblasts are established as the primary effector cells driving fibrosis, several recent studies have demonstrated that resident fibroblasts in the kidney also have a proinflammatory phenotype. Souma et al. reported that Epo-producing fibroblast–derived myofibroblasts upregulated the expression of the target genes of NFκB such as *Il6* and *Ccl2* in an UUO model [11]. Leaf et al. reported that renal pericytes could work as innate immune cells and respond to damage-associated molecular patterns (DAMPs) sensitively in the acute phase of renal injury [54]. In response to the stimulation with DAMPs, renal pericytes activated NLRP3 inflammasome and secreted IL-1β and IL-18 in a TLR2/TLR4 and Myd88-dependent manner and aggravated renal fibrosis in the IRI model. *In vitro*, Myd88 knockout pericytes and Tlr2/Tlr4 double knockout pericytes also reduced their ability to migrate and the expression of the genes associated with fibrosis in response to TGF-β or DAMPs. These results suggested that both the inflammatory and fibrogenic properties of pericytes were dependent on Myd88, TLR2, and TLR4. Further studies are necessary to elucidate the significance of proinflammatory fibroblasts in renal injury and CKD progression.

It is of note that an anti-inflammatory role of fibroblasts and pericytes was also reported. Using *FoxD1-Cre:CD73*$^{fl/fl}$ mice, Perry et al. demonstrated that CD73, an enzyme converting AMP to adenosine on fibroblasts and pericytes, was necessary to suppress inflammation and attenuate renal fibrosis after kidney injury [55]. Additionally, the absence of CD73 on the fibroblasts was associated with an increase in α-SMA expression *in vitro*. The authors hypothesized that the adenosine locally generated by CD73 on fibroblasts and pericytes might act on adenosine receptors in an autocrine and paracrine manner and attenuate macrophage infiltration and profibrotic properties. Importantly, renal fibroblasts can act as modulators of inflammation and contribute to normal tissue repair by interacting with the surrounding cells.

7. Age-dependent phenotype of fibroblasts: tertiary lymphoid tissue: associated fibroblasts

7.1 Tertiary lymphoid tissue formation in aged injured kidneys

An epidemiological study showed that elderly patients with AKI had an increased risk for CKD progression [56]. The mechanism for the maladaptive repair after AKI in the elderly remains unknown. To identify the mechanism, we compared the renal response to injury between young and aged mice. As in humans, while young kidney repaired itself after injury, aged kidney exhibited sustained tubular injury and fibrosis [8]. Unexpectedly, we found multiple TLTs in aged kidneys but not young kidneys in the chronic phase after kidney injury. TLT is an ectopic lymphoid tissue that develops at the site of chronic inflammation. TLTs are mainly composed of lymphocytes that are structurally and functionally supported by unique phenotypic fibroblasts inside TLTs (**Figure 1**). Unlike simple infiltration of the inflammatory cells, TLTs can promote lymphocyte proliferation and differentiation, resulting in the generation of antibody-secreting plasma cells, as recognized in secondary lymphoid organs [57]. Importantly, although TLTs are identified in various disease conditions, such as autoimmune diseases, infections, and cancers, TLTs can play beneficial or pathological roles in a context-dependent manner [58]. For example, in chronic inflammatory or autoimmune diseases, TLTs contribute to disease persistence and have detrimental effects on the host [59]. In contrast, during infections, TLTs are assumed to play beneficial roles to eliminate pathogens by promoting immune responses [60]. The role of TLTs in aged injured kidneys remains unclear, and will be discussed in the next section.

7.2 Characteristics and origin of fibroblasts inside tertiary lymphoid tissues

Fibroblasts inside TLTs exhibit unique characteristics that are distinct from those outside TLTs, such as the strong expression of p75NTR (**Figure 1**) [8]. After kidney injury, resident fibroblasts acquire the ability to produce RAs by upregulating RALDH2, which is assumed to promote the transition of the adjacent fibroblasts into p75NTR$^+$ TLT-associated fibroblasts. Some of the p75NTR$^+$ TLT-associated fibroblasts acquire abilities to secrete homeostatic chemokines such as CCL19 and CXCL13, which are the driving force for recruiting lymphocytes and promoting TLT

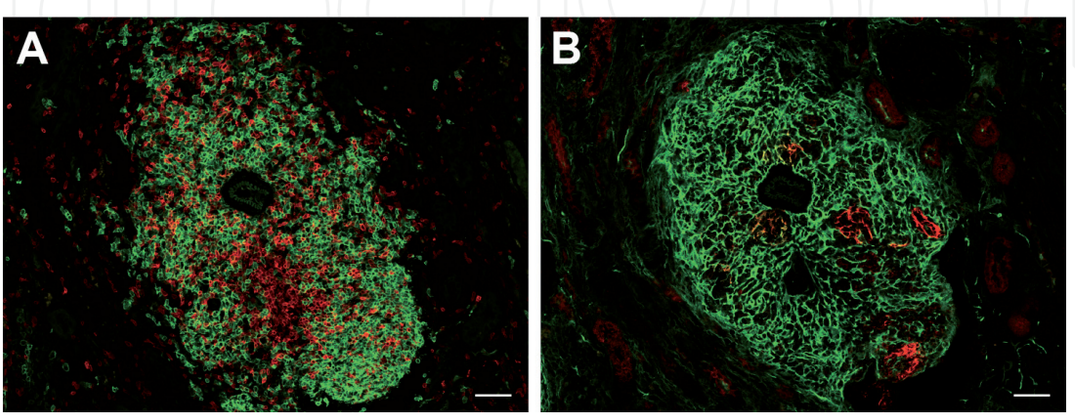

Figure 1.
Tertiary lymphoid tissues in aged murine kidney 45 days after ischemic reperfusion injury. (A) Immuno fluorescence staining of B220 (green) and CD3ε (red). Tertiary lymphoid tissues (TLTs) are mainly composed of B220$^+$ B cells and CD3ε$^+$ T cells. (B) Immunofluorescence staining of p75NTR (green) and CD21 (red). Fibroblasts inside TLTs express p75 neurotrophin receptor (p75NTR). In mature TLTs, some of the fibroblasts differentiate into p75NTR$^-$/CD21$^+$ follicular dendritic cells. Scale bar: 50 μm.

formation. Inside of more mature TLTs, $CD21^+/p75NTR^-$ follicular dendritic cells (FDCs) appear as part of stromal cells (**Figure 1**). FDCs are stromal cells residing in the B cell follicles of secondary lymphoid organs; they drive germinal center reactions [61]. These TLT-associated fibroblasts in the kidneys are *P0-Cre* lineage–labeled cells, suggesting that renal resident fibroblasts can differentiate into these various types of fibroblasts [8].

7.3 Clinical significance of tertiary lymphoid tissues in CKD and the elderly

Several studies have reported that TLTs are induced in various kidney diseases [62–65]. Additionally, we reported that TLTs developed not only in murine kidneys but also in human kidneys in an age-dependent manner [8]. In the analysis on kidneys from nephrectomy cases for renal cell carcinoma and autopsy, excluding pyelonephritis, glomerulonephritis, autoimmune kidney diseases, and hematological malignancies, TLTs were identified only in the elderly over 60 years old. The components of human TLTs are quite similar to those of murine TLTs. To evaluate TLTs objectively, we classified renal TLTs into three stages based on the immunostaining patterns as follows [66]. TLTs not containing $CD21^+$ FDCs or a germinal center response, dense $Ki67^+$ proliferative B cell clusters, were defined as stage 1. TLTs containing $CD21^+$ FDCs but no germinal center response were defined as stage 2. TLTs containing both $CD21^+$ FDCs and a germinal center response were defined as stage 3. In this classification, the severity of the TLT stages and the area of TLTs were related with the severity of ischemic injury in murine renal IRI models. In humans, more and higher-stage TLTs were identified in the kidneys of patients with CKD than without CKD among elderly patients 60 years or older in the analysis using kidneys from nephrectomy cases due to renal cell carcinoma [66]. These data demonstrated that the developmental stage of TLTs was associated with the severity of kidney injury, thereby indicating that TLTs have potential as a marker of severity of renal injury.

7.4 Potential of tertiary lymphoid tissues as therapeutic targets for CKD

Although TLTs are assumed to be associated with the severity of renal injury, it has been challenging to determine whether renal TLTs are pathogenic and if they directly affect renal function. We reported that, in unilateral renal IRI models of aged mice, the administration of GK1.5, anti-CD4 monoclonal antibody, diminished TLT formation and inflammatory marker expressions and improved renal fibrosis [8]. This result suggests that renal TLTs could be pathogenic and the therapies targeting renal TLTs thus have the potential to improve renal function in patients with CKD. As this intervention is not specific to TLTs and affects systemic immune systems, however, a more specific therapy for TLTs is necessary to determine whether TLTs in aged injured kidneys are detrimental or not.

8. Conclusion

Resident fibroblasts in the kidney are essential components to maintain homeostasis under physiological conditions. In CKD, dysfunction of renal fibroblasts causes the main pathological conditions of renal fibrosis, renal anemia, and peritubular capillary loss. Importantly, renal fibroblasts are heterogeneous and have the potential to change their phenotypes depending on the local microenvironment (**Figure 2**) [5]. Although myofibroblasts mainly contribute to renal fibrosis and deteriorate renal function by producing excessive ECM, they can also

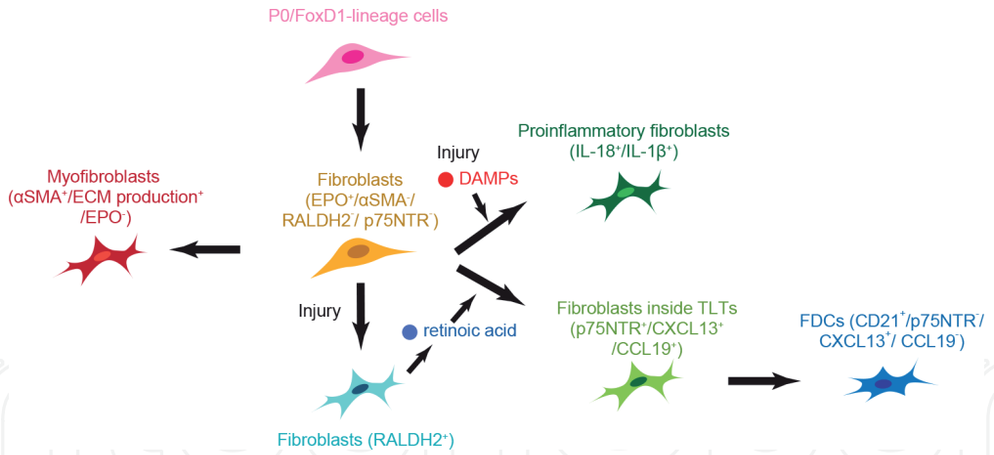

Figure 2.
Heterogeneity of fibroblasts in kidneys. Renal fibroblasts derive from P0- or FoxD1-Cre lineage–labeled cells. Renal fibroblasts change their phenotypes depending on the local microenvironment. Myofibroblasts have the potential to produce excessive extracellular matrix proteins at the cost of EPO production. In response to released DAMPs after injury, fibroblasts can differentiate into proinflammatory fibroblasts that produce IL-18 and IL-1β. In contrast, in aged kidneys, retinoic acids produced by activated fibroblasts after injury induce the differentiation of adjacent fibroblasts into p75NTR$^+$ fibroblasts, which produce homeostatic chemokines such as CXCL13 and CCL19, resulting in tertiary lymphoid tissue (TLT) formation. These chemokine-producing fibroblasts can further differentiate into CD21$^+$/p75NTR$^-$ FDCs in mature TLTs.

have host-protective roles in the early phase of kidney injury. Renal fibroblasts can differentiate into proinflammatory fibroblasts that secrete inflammatory cytokines and chemokines, which can promote TLT formation under several diseased conditions. Fibroblasts or pericytes also have an anti-inflammatory function via CD73 expression. A better understanding of the heterogeneity and roles of renal fibroblasts might lead to the development of a new therapeutic approach for kidney diseases. Recent novel technologies such as scRNA-seq have revealed the heterogeneity of renal fibroblasts that had not previously been identified by conventional technologies [26]. The application of these technologies to various clinical renal diseases is expected to further clarify the heterogeneity of renal fibroblasts, which will result in an enhanced understanding of the pathophysiology of kidney diseases and the development of novel treatments.

Conflict of interest

YS is employed by the TMK Project, which is a collaboration between Kyoto University and Mitsubishi Tanabe Pharma. MY receives research grants from Mitsubishi Tanabe Pharma and Boehringer Ingelheim. TY reports no conflicts of interest.

Appendices and nomenclature

CKD	chronic kidney disease
ESRD	end-stage renal disease
EPO	erythropoietin
TLT	tertiary lymphoid tissue
ECM	extracellular matrix
p75NTR	p75 neurotrophin receptor
P0	myelin protein zero
α-SMA	α-smooth muscle actin

scRNA-seq	single cell RNA-sequencing
MSC	mesenchymal stem cell
iDTR	inducible diphtheria toxin receptor
DT	diphtheria toxin
UUO	unilateral ureteral obstruction
AKI	acute kidney injury
TGFβ-1	transforming growth factor β-1
HIF	hypoxia-inducible factor
PHD	prolyl hydroxylase domain–containing protein
ESA	erythropoiesis-stimulating agent
SGLT2	sodium-glucose cotransporter 2
RALDH2	retinaldehyde dehydrogenase 2
RA	retinoic acid
RAR	retinoic acids receptor
DAMP	damage-associated molecular pattern
FDC	follicular dendritic cell

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